

1 Claims

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3 1. A method of producing a soluble bioactive
4 domain of a protein of interest, the method
5 comprising the step of selecting at least one
6 candidate soluble domain of the protein and
7 assessing the produced protein of each domain
8 for desired activity.

9

10 2. The method according to claim 1 comprising the
11 step of amplifying DNA encoding at least one
12 candidate soluble domain, cloning the amplified
13 DNA encoding each candidate domain into at
14 least one expression vector, using each of said
15 vectors into which the DNA has been cloned to
16 each transfect or transform one or more host
17 cell strains, expressing said DNA in one or
18 more of said host cell strains, and analysing
19 expression products from said host cells for
20 solubility.

21

22 3. The method according to claim 2 comprising
23 steps:

- 24 (a) analysing DNA coding for the protein of
25 interest to identify one or more candidate
26 soluble domains
27 (b) providing oligonucleotide primers to amplify
28 DNA encoding each domain
29 (c) amplifying said DNA with said primers
30 (d) cloning amplified DNA from step (c) for each
31 domain into at least one expression vector

- 1 (e) optionally screening clones for correct
- 2 orientation of DNA
- 3 (f) using each of the vectors of step (d) into
- 4 which the DNA has been cloned to each transfect
- 5 or transform one or more host cell strains,
- 6 (g) expressing said DNA in one or more of said host
- 7 cell strains, and
- 8 (h) analysing expression products from said host
- 9 cells for solubility.
- 10
- 11 4. The method according to claim 2 or claim 3
- 12 comprising the step of producing a soluble
- 13 bioactive protein domain of said protein of
- 14 interest.
- 15
- 16 5. The method according to any one of claims 2 to
- 17 4 wherein at least three candidate soluble
- 18 domains are selected and DNA is amplified for
- 19 each of said domains.
- 20
- 21 6. The method according to any one of claims 2 to
- 22 5 wherein said DNA encoding each selected
- 23 domain is amplified under at least two,
- 24 preferably at least three different PCR
- 25 programs in parallel.
- 26
- 27 7. The method according to claim 6 wherein said
- 28 PCR programs are selected from (i) a standard
- 29 PCR programme using a predicted annealing
- 30 temperature for the primers; (ii) a standard
- 31 PCR programme using a temperature in the range
- 32 48 to 52°C, preferably 50°C as the temperature

- 1 for annealing and (iii) a touchdown PCR
2 programme, where the annealing temperature
3 starts at a temperature in the range 62 to
4 67°C, preferably 65°C, and then gradually
5 decreases to a temperature in the range 48 to
6 52°C, preferably 50°C, over the subsequent
7 cycles.
8
- 9 8. The method according to any one of claims 2 to
10 7 wherein the amplified DNA encoding each
11 domain is cloned into a plurality of different
12 expression vectors.
13
- 14 9. The method according to claim 8 wherein the
15 plurality of vectors include one or more of a
16 vector capable of encoding a fusion protein
17 with a poly-Histidine tag, a vector capable of
18 conferring tight regulation of translation to
19 impose stringent expression conditions, a
20 vector capable of encoding a fusion protein
21 with a solubility enhancing tag.
22
- 23 10. The method according to claim 9 wherein the
24 solubility enhancing tag comprises a
25 glutathione-S-transferase tag, a dihydrofolate
26 reductase tag, a NusA tag or a SNUT tag.
27
- 28 11. The method according to any one of claims 2 to
29 10 wherein the vectors are each transfected or
30 transformed into a plurality of different host
31 cell strains
32

- 1 12. The method according to any one of claims 2 to
2 11 wherein the host cell strains are different
3 *E. coli* strains.
4
- 5 13. The method according to claim 12 wherein the *E*
6 coli strains are selected from
7 Rosetta(DE3)pLacI, Tuner(DE3)pLacI, Origami
8 BL21(DE3)pLacI and TOP10F'.
9
- 10 14. The method according to any one of claims 2 to
11 13 including the step of screening
12 transformants for correct orientation of DNA.
13
- 14 15. The method according to claim 14 wherein the
15 step of screening transformants for correct
16 orientation of the insert is performed using
17 dot-blotting.
18
- 19 16. The method according to any one of claims 2 to
20 14 wherein the expression products from said
21 host cells are analysed using ELISA or dot-
22 blotting methods.
23
- 24 17. The method according to any one of the
25 preceding claims wherein analysis of expression
26 products includes the use of chloroform and UV
27 light to stain protein on an SDS-PAGE gel.
28
- 29 18. The method according to claim 17, wherein the
30 method further comprises the subsequent use of
31 the chloroform-stained SDS-PAGE gel for western
32 blotting for the identification of proteins.

- 1 19. The method according to any one of the
2 preceding claims wherein the protein of
3 interest is a protein encoded by the yotiao
4 gene, the murine MAR1 protein or the human Jak1
5 protein.
6
- 7 20. A method of producing a soluble bioactive
8 domain of a protein of interest comprising the
9 steps:
10 (a) analysing DNA coding for the protein of
11 interest to identify one or more candidate
12 soluble domains
13 (b) providing oligonucleotide primers to
14 amplify DNA encoding each domain
15 (c) amplifying said DNA using, in parallel, a
16 standard PCR programme using a predicted
17 annealing temperature for the primers; (ii) a
18 standard PCR programme using a temperature in
19 the range 48 to 52°C, preferably 50°C, as the
20 temperature for annealing and (iii) a touchdown
21 PCR programme, where the annealing temperature
22 starts at a temperature in the range 62 to
23 67°C, preferably 65°C, and then gradually
24 decreases to a temperature in the range 48 to
25 52°C, preferably 50°C, over the subsequent
26 cycles.
27 (d) cloning amplified DNA from step (b) into a
28 plurality of different expression vectors,
29 (e) optionally screening clones for correct
30 orientation of DNA
31 (f) using each of the vectors of step (d) into
32 which the DNA has been cloned to each transfect

1 or transform a plurality of different host cell
2 strains
3 (g) expressing said DNA in one or more of said
4 host cell strains, and
5 (h) analysing expression products from said
6 host cells for solubility.

7
8 21. The method according to claim 20 wherein at
9 least three candidate soluble domains are
10 selected and DNA is amplified for each of said
11 domains.

12
13 22. The method according to claim 20 or claim 21
14 wherein the plurality of vectors include one or
15 more of a vector capable of encoding a fusion
16 protein with a poly-Histidine tag, a vector
17 capable of conferring tight regulation of
18 translation to impose stringent expression
19 conditions, a vector capable of encoding a
20 fusion protein with a solubility enhancing tag.

21
22 23. The method according to claim 22 wherein the
23 solubility enhancing tag comprises a
24 glutathione-S-transferase tag, a dihydrofolate
25 reductase tag, a NusA tag or a SNUT tag.

26
27 24. The method according to any one of claims 20 to
28 23 wherein the host cell strains are different
29 *E. coli* strains.

30
31 25. The method according to claim 24 wherein the *E*
32 *coli* strains are selected from

- 1 Rosetta(DE3)pLacI, Tuner(DE3)pLacI, Origami
2 B21(DE3)pLacI and TOP10F.
3
- 4 26. A soluble bioactive domain of a protein
5 produced by the method according to any one of
6 claims 1 to 25.
7
- 8 27. Use of a sortase gene product as a purification
9 tag.
10
- 11 28. The use according to claim 27 wherein the
12 sortase gene product is a Staphylococcus aureus
13 srtA gene product.
14
- 15 29. The use according to claim 27 or claim 28
16 wherein the sortase gene product is encoded by
17 the nucleotide sequence shown in Figure 8 or a
18 variant or fragment thereof.
19
- 20 30. The use according to any one of claims 27 to 29
21 wherein the sortase gene product comprises
22 amino acids 26 to 171 of the SrtA sequence
23 shown in Figure 8 or a variant or fragment
24 thereof.
25
- 26 31. An expression construct for the production of
27 recombinant polypeptides, which construct
28 comprises an expression cassette consisting of
29 the following elements that are operably
30 linked: a) a promoter; b) the coding region of
31 a DNA encoding a sortase gene product as a
32 purification tag sequence; and c) a cloning

- 1 site for receiving the coding region for the
2 recombinant polypeptide to be produced; and d)
3 transcription termination signals.
4
- 5 32. The expression construct according to claim 31
6 wherein the sortase gene product is a
7 *Staphylococcus aureus* srtA gene product.
8
- 9 33. The expression construct according to claim 31
10 or claim 32 wherein the sortase gene product is
11 encoded by the nucleotide sequence shown in
12 Figure 8 or a variant or fragment thereof.
13
- 14 34. The expression construct according to any one
15 of claims 31 to 33 wherein the sortase gene
16 product comprises amino acids 26 to 171 of the
17 SrtA sequence shown in Figure 8 or a variant or
18 fragment thereof.
19
- 20 35. A method for producing a polypeptide,
21 comprising: a) preparing an expression vector
22 for the polypeptide to be produced by cloning
23 the coding sequence for the polypeptide into
24 the cloning site of an expression construct as
25 claimed in any one of claims 30 to 34; b)
26 transforming a suitable host cell with the
27 expression construct thus obtained; and c)
28 culturing the host cell under conditions
29 allowing expression of a fusion polypeptide
30 consisting of the amino acid sequence of the
31 purification tag with the amino acid sequence
32 of the polypeptide to be expressed covalently

- 1 linked thereto; and d) isolating the fusion
2 polypeptide from the host cell or the culture
3 medium by means of binding the fusion
4 polypeptide present therein through the amino
5 acid sequence of the purification tag.
6
- 7 36. The method according to claim 35, wherein the
8 sortase gene product is a *Staphylococcus aureus*
9 *srtA* gene product.
10
- 11 37. The method according to claim 35 or claim 36
12 wherein the sortase gene product is encoded by
13 the nucleotide sequence shown in Figure 8 or a
14 variant or fragment thereof.
15
- 16 38. The method according to any one of claims 37 to
17 35 wherein the sortase gene product comprises
18 amino acids 26 to 171 of the *SrtA* sequence
19 shown in Figure 8 or a variant or fragment
20 thereof.
21
- 22 39. A fusion polypeptide obtained by the method of
23 any one of claims 35 to 38.
24
- 25 40. A purification tag comprising a sortase gene
26 product.
27
- 28 41. The purification tag according to claim 40
29 wherein the gene product is a *Staphylococcus*
30 *aureus srtA* gene product.
31

- 1 42. The purification tag according to claim 40 or
2 claim 41 wherein the sortase gene product is
3 encoded by the nucleotide sequence shown in
4 Figure 8 or a variant or fragment thereof.
5
- 6 43. The purification tag according to any one of
7 claims 40 to 42 wherein the sortase gene
8 product comprises amino acids 26 to 171 of the
9 SrtA sequence shown in Figure 8 or a variant or
10 fragment thereof.
11
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